

## ***Interactive comment on “Depolymerization and mineralization – investigating N availability by a novel $^{15}\text{N}$ tracing model” by Louise C. Andresen et al.***

**Anonymous Referee #3**

Received and published: 6 April 2016

In the present manuscript "Depolymerization and mineralization – investigating N availability by a novel  $^{15}\text{N}$  tracing model", Andresen et al. compared an analytical and a numerical approach for estimating gross rates of peptide depolymerization, amino acid immobilization, and (amino acid) N mineralization in two forest soils. Current knowledge about soil organic N dynamics is particularly limited, in part due to technical challenges, and thus studies of amino acid turnover are essential to our understanding of N cycling. All methods and models have their limitations, and further improvement of such analytical approaches is paramount for more accurate estimations of gross N dynamics in soils. In the current manuscript, the authors propose that their numerical approach provides more robust and coherent estimations of gross production and con-

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sumption rates compared to the “traditional” analytical approach developed by Kirkham and Bartholomew (1954). However, the work in its present form is not convincing, as it has a fundamental experimental flaw and inconclusive reasoning.

My main concern relates to the high amount of  $^{15}\text{N}$ -labelled AA to both soils: approx. 600% and 120% of the initial FAA pool was added as  $^{15}\text{N}$ -AA to the Podzol and Umbrisol, respectively (based on data given in Table 1). It is known that addition of a given substrate often stimulates its consumption, and thus it is common practice to add only a small fraction of the initial target pool when performing isotope pool dilution assays, in order to minimize such bias on gross rate estimates. Furthermore, the authors erroneously state “Following the recommendation by Wanek et al. (2010), the FAA label addition was 10 to 20 times larger than the initial FAA content in the original substrate (litter or soil)” (Pg 9 L25-26). However, Wanek et al. (2010) clearly state that a maximum of 25% of the initial FAA pool should be added in the form of  $^{15}\text{N}$ -tracer, based on a preliminary determination of the size of the FAA pool. The excessive addition of  $^{15}\text{N}$ -AA in the present study likely resulted in biased gross rate estimates, in particular CFAA, and thus also affected the calculation of nitrogen use efficiency (NUE). The stimulation of amino acid mineralization rates due to high  $^{15}\text{N}$ -AA label addition, are also likely to have resulted in an overestimation of MFAA rates with the numerical approach (and not only rates estimated by the analytical approach), which would explain the high MFAA rates estimated. In Figure 2d and 3d, it becomes also evident that the  $^{15}\text{N}$ -AA label was not homogeneously distributed, otherwise there would have not been an increase in  $^{15}\text{N}/^{14}\text{N}$  in the first time interval. Of course, for this time interval, it is not possible to calculate a rate using the analytical approach. The weak dilution of the  $^{15}\text{N}$ -AA label during the first time intervals was not due to low depolymerization rates, as suggest by the authors, but rather likely due to high  $^{15}\text{N}$ -labelled substrate addition, which resulted in an enrichment of the FAA pool of 60-70 at%. In line with the comment by Referee #1, I thus think that it is necessary to repeat the experiment with appropriate  $^{15}\text{N}$ -labelled substrate addition.

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This brings me to the question of how to evaluate which approach yields more realistic rate estimates, which certainly cannot be concluded by simply comparing rates estimated by the two different approaches. I believe that both analytical and numerical models have disadvantages and limitations. For instance, I agree with Referee #1 and #2 that the current numerical model is missing a microbial biomass pool with a different turnover time than the SON pool. The authors also claim that their numerical model overcomes the problem of high label addition by integrating gross rates over a longer time. As already pointed out by Referee #1, the enormous  $^{15}\text{N}$ -AA input could result in end-product inhibition of peptidases, leading to lower depolymerization rates during longer incubations. Here, I would like to add that longer soil incubations often result in ammonium accumulation over time, due to the absence of plant roots, which would constantly remove a part of the soil nutrients. For example, such an ammonium accumulation was observed for the Podzol, as the ammonium concentration increased 3-fold during the 240h incubation (Figure 2a). Therefore, I suggest that the authors need to provide some experimental evidence that the numerical model actually overcomes problems such as high label addition. Regarding the comparison between the two different approaches, statistical support should be provided for differences in rates estimated by the two models.

Furthermore, the authors claim that using gross rates computed by their numerical model (IFAA and MFAA) yields more accurate estimates of microbial nitrogen use efficiency compared to the model by Mooshammer et al. (2014). The authors raise two points: (1) the analytical approach yields less accurate rate estimates (but see comment above), and (2) Mooshammer et al. (2014) use gross N mineralization (M) instead of amino acid mineralization (MFAA). However, the nitrogen use efficiency model proposed in the present study (NUEFAA) is conceptually different than that by Mooshammer et al. (2014) (NUE). The authors estimate here amino acid-N use efficiency (NUEFAA) based on amino acid immobilization and amino acid mineralization, whereas Mooshammer et al. based their model on gross amino acid consumption rate as proxy for microbial organic N uptake, since proteins are the main N-containing

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compounds in soil and plant litter. Therefore, both NUEFAA and NUE models are conceptually justified and there is no evidence that using MFAA instead of M yields better estimates of microbial NUE. Indeed, estimates of microbial NUE could be improved by including also organic N compounds other than amino acids, which, however, remains a great analytical challenge.

I also suggest that the authors should more carefully prepare the manuscript, as there are some inconsistencies and mistakes. Equations of the analytical approach (Eq. 1, 2 and 3) are partially wrong: equation 1 for production rate has a mistake in the numerator of the second term; equation 2 for the consumption rate is actually the production rate; and, equation 3, for the case when there is no change in concentration over time, also seems wrong, at least when compared to their former work (Andresen et al. 2015). Furthermore, the authors used different units:  $\mu\text{g N/g}$ ,  $\mu\text{g FAA/g}$ ,  $\mu\text{mol N/g}$ ,  $\text{nmol N/g}$ . In some instances, it is not even stated whether it refers to N or FAA (Table 1, Table 2). I suggest using consistent units throughout the manuscript. As I understood it, the initial FAA concentrations presented in Table 1 should correspond to the sum of FAA presented in Figure 4. For Podzol, Table 1 shows  $1.3 \mu\text{g}$ , whereas in Figure 4 the values roughly sum up to  $3 \mu\text{g}$ . In turn, for Umbrisol, both Table 1 and Figure 4 seems consistent:  $7.7 \mu\text{g}$  in Table 1, as well as roughly  $7.7$  in Figure 4.

Specific comments:

Pg 1 L14: Comma is missing before which

Pg 1 L16: Delete "2)"

Pg 2 L13: correct to "reaches"

Pg 2 L14: From a biological rather than mathematical perspective, I would say that low NUE leads to high N mineralization, and not that high N mineralization leads to low NUE.

Pg 2 L23-24: The authors should state what the obvious limitations are.

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Pg 4 L1-2: For N mineralization, 13 min for equilibration between added and native ammonium is quite short.

Pg 4 L3-4: Why were FAAs also extracted with 1 M KCL?

Pg 4: The authors used the protocol developed by Wanek et al. (2010). However, the authors do not even once cite this work in Materials & Methods.

Pg 4 L16: The reference is wrong. It should be Husek, 1991.

Pg 4 L18-23: The authors should explain why some amino acids have the same m/z: Alanine and Glycine (m/z 116/117); Leucine, Serine, Isoleucine and Threonine (m/z 158/159); Proline and Aspartic acid (m/z 142/143);

Pg 5 L5 and L15: The original references (Kirkham and Bartholomew, 1954; Watkins and Barraclough, 1996) are sufficient. Delete reference Andresen et al., 2015.

Pg 5 L13: Specify that it is excess  $^{15}\text{N}$  abundance.

Pg 6 L15: I do not always see a good fit of the model in Figure 2 and 3. For example in Figure 3b, the fit of the model for ammonium concentration seems not to fit the experimental data.

Pg 8 L18-19: In Wanek et al. (2010) the samples were plant litter and not organic soil.

Figure 1 B: Equation is wrong.

Table 1: The initial soil ammonium concentrations of both soils should be stated. There is no need to say that the C:N ratio refers to dry soil.

Figure 4: Typo on the y-axis.

#### References

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Kirkham DON, Bartholomew WV. (1954). Equations for following nutrient transformations in soil, utilizing tracer data. *Soil Sci Soc Am J* 18: 33–34.

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Wanek W, Mooshammer M, Blöchl A, Hanreich A, Richter A. (2010). Determination of gross rates of amino acid production and immobilization in decomposing leaf litter by a novel  $\text{N-15}$  isotope pool dilution technique. *Soil Biol Biochem* 42: 1293–1302.

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Interactive comment on *SOIL Discuss.*, doi:10.5194/soil-2016-11, 2016.

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